

The University of Chicago

ELECTRODIALYSIS OF SEEDS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1942

By

JOHN D. NELLY

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(WITH TWO FIGURES)

Introduction

The study herein reported was undertaken with the purpose of learning something of the effects of electrolysis on a living organism. When electrolysis takes place in living tissues in the presence of membranes the process can more properly be called electrodialysis. The earliest work on the electrodialysis of plant material was that of LISBONNE and VULQUIN (3, 4) in which they reported the inactivation of malt amylase after electrodialysis at 110 volts d.c. under sterile conditions for periods of five to twelve days. Treatment of this inactivated amylase with such salts as NaH_2PO_4 , Na_2HPO_4 , and KNO_3 partially restored its hydrolyzing power. Before the work of LISBONNE and VULQUIN it was thought that animal and plant amylases were essentially different because animal amylases could be inactivated by simple dialysis while plant amylases did not lose their hydrolytic power when subjected to the same process. The essential difference lay in the fact that plant amylases needed something more powerful than ordinary dialysis to free them of their electrolytes.

The conventional type of electrodialysis cell consists of a cell divided into three compartments by means of two differentially permeable membranes. Electrodes are placed in the end compartments and the suspension to be electrodialyzed is placed in the middle compartment. Electrolytes which can pass through the membrane pass out into the end chambers under the influence of the electrical potential. Ions with positive charges on them go to the cathode chamber while negative ions pass into the anode chamber. A colloidal suspension may be purified in this manner by the process of losing electrolytes to the anode and cathode compartments of the electrodialysis cell while the colloidal material remains in the middle chamber. This method may be used to free gelatin of most of its inorganic matter and to remove adsorbed bases from clay. In the case of living tissues such structures as cell membranes and seed coats determine which electrolytes shall pass through them.

Diastase from pancreatic juice of the cow was electrolyzed by MAIGNON (6) in a 16-mm. U-tube at 110 volts d.c. for as long as six days. Prolonged dialysis resulted in a loss of diastatic activity, which MAIGNON attributed to dissociation of organo-mineral groupings in the diastase and not to the formation of such substances as hypochloric or persulphuric acids at the electrodes. MOORE, REEVES, and HIXON (10) used electrodialysis in a study of Jonathan spot of apples. They claimed that the dialysate of apple parings with Jonathan spot was higher in basic than acidic constituents, while that from normal apple parings showed equivalent amounts of acid and base. A

study of the effects of electrodialysis has been published by MULLISON (11), in which he reported loss of ash constituents from the seeds, decreased germination, and stunted growth of plants from electrodialyzed Alaska pea seeds. Electrodialysis was employed by MAKSIMOW (7) on legume seeds, grain seeds, oil seeds, and tobacco. He showed that partial demineralization reduced the germination of legume and oil seeds but not of grain seeds. SEIFRIZ (13) has demonstrated the breakdown of plant tissue under the influence of an electric current. He placed the tissue under consideration in a dish partially filled with 0.1 N sodium chloride solution, inserted a platinum electrode into the tissue, and had the other platinum electrode in the surrounding sodium chloride solution. Through this he allowed a 110-volt direct current to pass for 18–24 hours. When the cathode was in the tissue a very marked breakdown of the tissue occurred along with a great increase in pH in the vicinity of the electrode. When the anode was in the tissue, breakdown was slight and the pH was not lowered to any great extent. SEIFRIZ attributed breakdown at the cathode to autolysis favored by reducing conditions. After autolysis oxidation would be favored by the highly alkaline condition of the cathode.

Materials and methods

Electrodialysis was carried out in a Mattson electrodialysis cell, using parchment paper membranes, a copper grid cathode, and a carbon anode. A direct current of 80 to 150 volts was supplied by either a rectifier or a direct current line. The rectifier was equipped with a rheostat for regulating voltage, while the voltage from the direct current line was controlled by means of a resistance coil arranged in a circuit as a potentiometer. Temperature of the cell was controlled by running water through glass coils placed in the end chambers. Although no attempt was made at rigid control of the temperature, this cooling system kept the temperature of the cell near 24° C. Before being placed in the cell the seeds were given a preliminary soaking for either five or six hours in enough distilled water to allow maximum swelling for that length of time. During electrodialysis the seeds were suspended in a cheesecloth bag in the middle chamber of the cell. After the cell was filled with distilled water and the seeds placed in position a steady potential was maintained until the current increased to 450 milliamperes. When the current approached this value, it was turned off and the liquid in the end chambers was replaced with distilled water.

Subsequent to treatment seeds were placed in flannel roll type seed testers and kept at 20° C. for germination. Germination counts were made at the end of either three or five days.

Carbon dioxide production of germinating seeds was determined by absorption in barium hydroxide in Pettenkoffer tubes.

For mineral analyses the seeds were ashed whole in large crucibles in a muffle furnace at temperatures between 500° and 550° C. Seeds which had been electrolyzed for periods of an hour or longer were extremely hard to

ash and had to be treated with nitric and hydrochloric acids to induce ashing. Apparently the ashing process is catalyzed by the presence of some substance or substances lost during the electrodialysis process. After dissolving the ash in hydrochloric acid it was used in the determination of magnesium, calcium, and potassium. Calcium was determined volumetrically by precipitating as the oxalate and titrating with standard potassium permanganate. For magnesium the gravimetric method of weighing magnesium pyrophosphate was employed. The perchloric acid method was used in the determination of potassium (14). Before the potassium determinations were made, such interfering substances as aluminum, calcium, phosphates, and sulphates were removed by the method described by SCOTT (12).

Ascorbic acid was determined in the germinating seeds by the titration method, using the dye 2,6 dichlorophenolindophenol. Extracts from the seeds were made by triturating them with ignited acid-washed sand in a solution of 3 per cent. trichloroacetic and 2 per cent. metaphosphoric acid. This method of determining ascorbic acid is outlined in full by MACLINN and FELLERS (5). Standardization of the dye was carried out by the method of MENAKER and GUERRANT (9), using sodium thiosulphate.

Results

ELECTRODIALYSIS AND GERMINATION

One of the most striking effects of electrodialysis on seeds is the inhibition of germination. Seeds were soaked in distilled water before being placed in the electrodialysis cell. The Laxton's Progress pea seeds were given a preliminary soaking of five hours, while the Alaska peas and red kidney bean seeds were given a preliminary soaking of six hours. After the period of treatment, both control and test seeds were placed in flannel roll type seed testers soaked in tap water and were kept at 20° C. for germination. The effects of electrodialysis on the germination of pea and red kidney bean seeds are summarized in tables I to IV.

Seeds which failed to germinate, whether electrodialyzed or control seeds, were always infected with bacteria and sometimes with fungi. Con-

TABLE I

EFFECT OF ELECTRODIALYSIS AT 150 VOLTS ON THE GERMINATION OF LAXTON'S PROGRESS PEA SEEDS

PERIOD OF TREATMENT	CONTROL		ELECTRODIALYSIS	
	NUMBER OF SEEDS USED	PERCENTAGE OF GERMINATION	NUMBER OF SEEDS USED	PERCENTAGE OF GERMINATION
<i>min.</i>		%		%
15	80	90	80	59
30	223	81	224	26
60	223	83	224	18
90	203	93	200	7
120	21	86	20	0
180	140	85	140	2

TABLE II

EFFECT OF ELECTRODIALYSIS AT 110 VOLTS ON THE GERMINATION
OF ALASKA PEA SEEDS

SAMPLE NUMBER	PERCENTAGE OF GERMINATION					
	1 HOUR		2 HOURS		3 HOURS	
	CONTROL	ELECTRO- DIALYSIS	CONTROL	ELECTRO- DIALYSIS	CONTROL	ELECTRO- DIALYSIS
	%	%	%	%	%	%
1	84	62	83	51	90	27
2	94	66	90	47	92	32
3	80	50	84	16	86	29
Number of seeds used	330	330	330	330	330	330

TABLE III

EFFECT OF ELECTRODIALYSIS AT 80 VOLTS ON THE GERMINATION
OF ALASKA PEA SEEDS

SAMPLE NUMBER	PERCENTAGE OF GERMINATION					
	1 HOUR		2 HOURS		3 HOURS	
	CONTROL	ELECTRO- DIALYSIS	CONTROL	ELECTRO- DIALYSIS	CONTROL	ELECTRO- DIALYSIS
	%	%	%	%	%	%
1	91	39	83	33	84	7
2	78	47	86	35	89	12
3	82	51	90	31	81	7
Number of seeds used	330	330	330	330	330	330

TABLE IV

EFFECT OF ELECTRODIALYSIS AT 80 VOLTS ON THE GERMINATION
OF RED KIDNEY BEAN SEEDS

SAMPLE NUMBER	PERCENTAGE OF GERMINATION			
	1 HOUR		2 HOURS	
	CONTROL	ELECTRO- DIALYSIS	CONTROL	ELECTRO- DIALYSIS
	%	%	%	%
1	93	26	98	13
2	84	25	98	8
3	95	37	94	4
4	89	20	81	9
5			87	26
6			94	6
Number of seeds used	220	220	330	330

trol lots never had fungi growing on the sprouted seeds. Presence of fungi on sprouted electrodialed seeds was somewhat common. Thus it can be seen that some change which takes place during electro dialysis makes the seed a better growth medium for fungi.

EFFECTS OF ELECTRODIALYSIS ON CARBON DIOXIDE PRODUCTION

Because of the fact that electro dialysis had such a marked effect on germination it was decided that a study should be made to learn whether or not this reduced germination was accompanied with a change in respiratory activity. For these determinations weighed samples of about sixty seeds of Laxton's Progress pea were used. Each lot of seeds was soaked for five hours in 100 ml. of distilled water at room temperature before it was placed in the electro dialysis cell. When the sample to be electro dialyzed was placed in the cell the water in which the control sample had been soaking was poured off and fresh water added to the beaker containing the control seeds.

For this part of the work the seeds were electro dialyzed at 150 volts. At the end of the dialysis period the seeds were placed in the respiration apparatus in order to determine their rates of carbon dioxide production. The time during which the seeds were left in the carbon dioxide apparatus was usually about one and one-quarter hours. Production rate of carbon dioxide is expressed in milligrams per hour per gram of seed. The weight basis for this expression is the air dry weight of the seeds at the beginning of the experiment. After the initial carbon dioxide determination the seeds were placed in flannel rolls moistened in tap water and kept at 20° C. The seeds were removed from the rolls long enough each following day to determine their rates of carbon dioxide production.

Seeds which are treated by the electro dialysis process are especially susceptible to attack by microorganisms. It is conceivable that these microorganisms might greatly alter the carbon dioxide production. At the end of 24 hours there was no evidence of infection, but it could be noticed on the second day after electro dialysis. In order to discourage growth of the infecting organisms, both treated and control seeds were soaked 3 minutes in a 0.25 per cent. sodium hypochlorite solution and rinsed in tap water before being placed in the respiration chambers on the second and following days. By this treatment the seeds were kept in a sound condition; otherwise, they would have been disintegrated at the end of a few days.

Rates of carbon dioxide production for the variously treated seeds are indicated in table V. The means of the results presented in table V are plotted in figure 1. A marked difference in carbon dioxide production of electro dialyzed and control seeds is seen. Together with this lesser carbon dioxide production is found a marked decrease in the germinating percentage of the seeds. Germination, however, is not directly proportional to carbon dioxide production of the seeds. For instance, the seeds electro dialyzed 30 minutes have about thirteen times the germination percentage of seeds treated for 180 minutes, while the carbon dioxide production of the

former never becomes much more than twice that of the latter. It seems that seeds lose their ability to germinate but can not be killed because they still retain their respiration in part. Both the 90-minute and the 180-minute control lots of seeds exhibit much higher initial rates of respiration than did the 30-minute and 60-minute controls. This may possibly be explained by the fact that they were in water for longer periods than the other controls

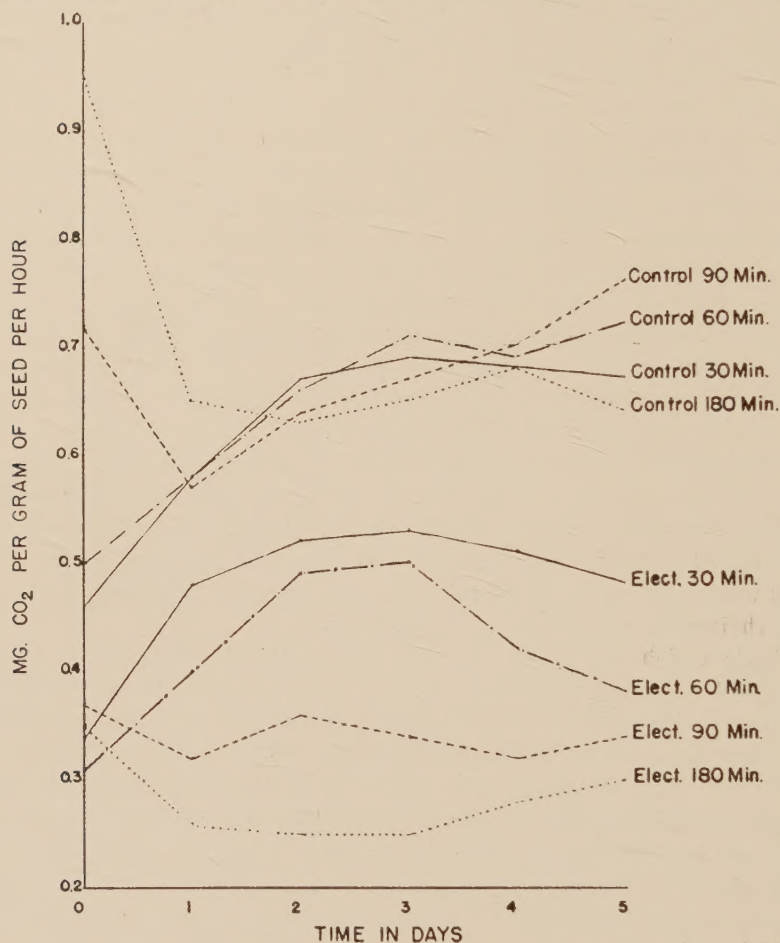


FIG. 1. Graphical representation of effects of electro dialysis on carbon dioxide production of pea seeds. Curves were plotted from means of results presented in table V.

and that increased moisture content may account for the higher respiratory rate. It is known that respiration in seeds increases with an increase in moisture content.

AMOUNTS OF ACID AND ALKALI REMOVED IN ELECTRODIALYSIS

In MATTSON's work (8) with the electro dialysis of soils and in the work of MOORE, REEVES, and HIXON (10) on the electro dialysis of apple parings,

TABLE V

RATES OF CARBON DIOXIDE PRODUCTION IMMEDIATELY AFTER AND AT ONE- TO FIVE-DAY INTERVALS AFTER ELECTRODIALYSIS. SEEDS WERE ELECTRODIALYZED FOR PERIODS OF 30, 60, 90, AND 180 MINUTES. BOTH CONTROL AND ELECTRODIALYZED SAMPLES WERE RUN IN DUPLICATE OR TRIPPLICATE. DETERMINATIONS WERE MADE AT 20° C.

TIME PERIODS	MG. CO ₂ PER GM. OF SEED PER HOUR							
	30 MINUTES		60 MINUTES		90 MINUTES		180 MINUTES	
	CON-TROL	ELEC-TRODI-ALYSIS	CON-TROL	ELEC-TRODI-ALYSIS	CON-TROL	ELEC-TRODI-ALYSIS	CON-TROL	ELEC-TRODI-ALYSIS
Beginning ...	0.47	0.37	0.47	0.33	0.74	0.40	0.96	0.35
	0.47	0.34	0.49	0.28	0.74	0.35	0.93	0.35
	0.44	0.32	0.54	0.33	0.69	0.35		
1st day	0.59	0.49	0.55	0.40	0.54	0.33	0.65	0.27
	0.60	0.50	0.56	0.36	0.60	0.32	0.64	0.24
	0.54	0.44	0.64	0.43	0.57	0.32		
2nd day	0.71	0.51	0.63	0.47	0.65	0.34	0.64	0.26
	0.65	0.51	0.65	0.48	0.63	0.36	0.62	0.23
	0.66	0.55	0.69	0.52	0.65	0.38		
3rd day	0.68	0.47	0.64	0.48	0.75	0.32	0.68	0.25
	0.70	0.52	0.78	0.52	0.59	0.31	0.62	0.24
	0.70	0.61			0.67	0.39		
4th day	0.71	0.47	0.68	0.43	0.72	0.31	0.70	0.26
	0.65	0.51	0.71	0.45	0.67	0.33	0.65	0.29
	0.69	0.54	0.69	0.37				
5th day	0.66	0.43	0.73	0.42	0.77	0.34	0.66	0.30
	0.70	0.45	0.70	0.38	0.74	0.34	0.62	0.29
	0.65	0.56	0.73	0.35				

it was noticed that the amount of alkali removed from the soil or diseased parings exceeded the amount of acid removed from them. Because of these findings it was decided to measure the amounts of acid and alkali removed from pea seeds and red kidney bean seeds.

One hundred and ten Alaska pea seeds were soaked for 6 hours in distilled water and then placed in the electrodialysis cell at 80 volts. At the

TABLE VI

ACID AND BASE EQUIVALENTS OF CATHODE AND ANODE SOLUTIONS FROM THE ELECTRODIALYSIS AT 80 VOLTS OF 110 ALASKA PEA SEEDS

INTERVAL	SAMPLE NUMBER	ML. 0.1 N HCl TO NEUTRALIZE CATHODE FLUID	ML. 0.1 N NaOH TO NEUTRALIZE ANODE FLUID
		<i>ml.</i>	<i>ml.</i>
0-40 minutes	1	5.95	1.44
	2	5.70	1.23
	3	3.46	1.10
40-80 minutes	1	2.91	1.05
	2	3.48	0.99
	3	3.02	1.05
80-120 minutes	1	2.38	1.05
	2	3.02	1.16
	3	2.92	1.05

TABLE VII

ACID AND BASE EQUIVALENTS OF CATHODE AND ANODE SOLUTIONS FROM THE ELECTRODIALYSIS AT 80 VOLTS OF 55 RED KIDNEY BEANS

INTERVAL	SAMPLE NUMBER	ML. 0.1 N HCl TO NEUTRALIZE CATHODE FLUID	ML. 0.1 N NaOH TO NEUTRALIZE ANODE FLUID
		<i>ml.</i>	<i>ml.</i>
0-40 minutes	1	4.80	1.11
	2	5.13	0.95
	3	3.46	0.83
40-80 minutes	1	4.95	1.00
	2	5.95	1.03
	3	4.72	1.11
80-120 minutes	1	4.96	1.28
	2	6.05	1.33
	3	4.48	1.39

end of 40, 80, and 120 minutes the liquids in the end chambers were drawn off, boiled, and titrated with standard sodium hydroxide or hydrochloric acid in order to determine the acid and base equivalents of materials removed from the seeds. The amounts of acid and base removed from Alaska pea seeds are indicated in table VI.

Red kidney beans were electrodialed at 80 volts in groups of 55 after being given a 6-hour soaking in distilled water. The amounts of acid and alkali removed from the kidney beans are shown in table VII.

TABLE VIII

ACID AND BASE EQUIVALENTS OF CATHODE AND ANODE SOLUTIONS FROM THE ELECTRODIALYSIS AT 80 VOLTS OF 100 LAXTON'S PROGRESS PEA SEEDS

INTERVAL	SAMPLE NUMBER	ML. 0.1 N HCl TO NEUTRALIZE CATHODE FLUID	ML. 0.1 N NaOH TO NEUTRALIZE ANODE FLUID
		<i>ml.</i>	<i>ml.</i>
0-40 minutes	1	8.00	1.83
	2	14.65	3.33
	3	19.10	4.50
	4	12.30	3.08
	5	10.03	1.94
40-80 minutes	1	12.50	3.00
	2	16.05	4.00
	3	17.83	4.90
	4	17.90	4.88
	5	16.10	3.66
80-120 minutes	1	14.70	3.90
	2	15.75	5.00
	3	13.35	4.60
	4	16.30	5.43
	5	15.55	4.13
120-160 minutes	4	8.96	4.66
	5	11.22	4.30
160-200 minutes	4	5.73	3.66
	5	6.54	3.50

Results similar to those obtained with Alaska pea and red kidney bean were obtained with Laxton's Progress pea seeds. These seeds were soaked in lots of 100 for 6 hours and electrodialyzed at 80 volts. Acid and base equivalents of the liquids in the end chambers of the electrodialysis cell after varying periods of treatment are shown in table VIII.

From the tables it can be seen that the bases of the cathode chamber are much more than equivalent to the acids of the anode chamber.

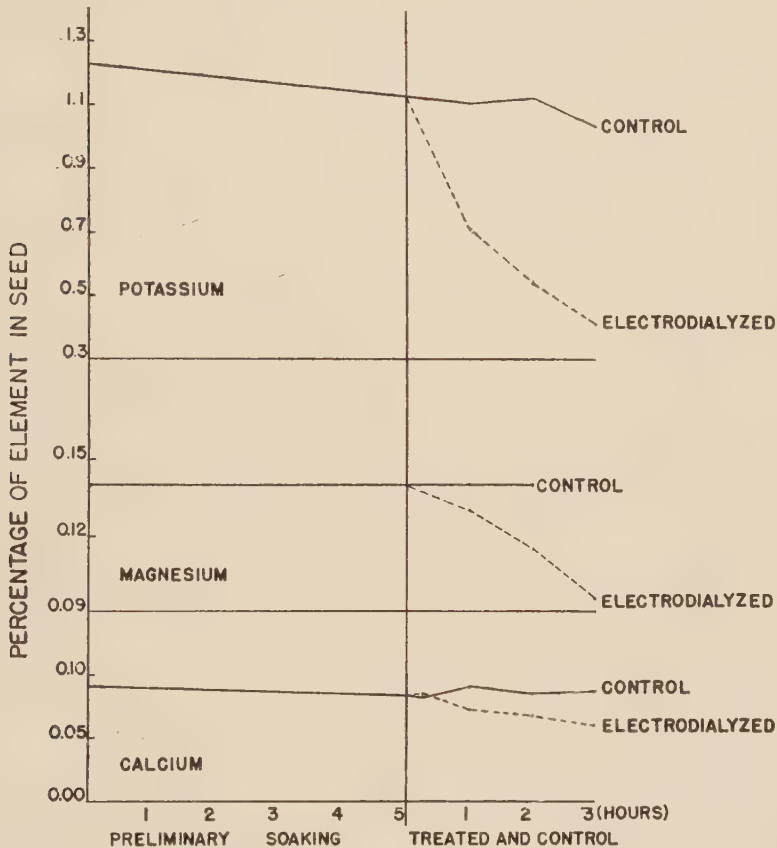


FIG. 2. Percentage of Ca, Mg, and K in seeds after the 5-hour preliminary soaking and after electrodialysis periods varying from 15 minutes to 3 hours.

LOSS OF MINERALS

A study was made of the loss of minerals from seeds during electro-dialysis. The seeds used for this part of the work were Laxton's Progress pea seeds. A potential of 150 volts was maintained across the electrodes of the electrodialysis cell. When the current reached 450 milliamperes it was stopped long enough to permit changing of the water in the end chambers of the cell. Seeds were given a 5-hour soaking in distilled water before being placed in the cell. Control lots were given a change of distilled water at the end of the preliminary 5-hour period and were allowed to remain in the

beaker of water as long as the corresponding lot of seeds was in the electro-dialysis cell.

At the end of the electro-dialysis period both control and treated seeds were placed in a 100° C. oven and dried to be kept for analysis. Methods of analysis have been mentioned earlier.

The calcium, magnesium, and potassium contents of the seeds after different treatments are tabulated in table IX. These elements were determined by analysis of the ash from the seeds. The fact that these elements could be removed from the seeds indicates that they must occur in the ionic form in the soaked pea seed. Magnesium and calcium were not lost to any appreciable extent by diffusion from the control lots of seeds; potassium diffused out in somewhat greater quantities. Each of these elements was removed in much greater quantities by electro-dialysis. Calcium, magnesium, and potassium contents of the seeds after different treatments are shown in figure 2.

TABLE IX

COMPOSITION OF PEA SEEDS AFTER HAVING BEEN SUBJECTED TO DIFFERENT TIMES OF ELECTRODIALYSIS. THE AIR DRY WEIGHT OF THE SEED AT THE BEGINNING OF THE EXPERIMENT IS USED AS THE BASIS FOR PERCENTAGE CALCULATIONS

TREATMENT	PERCENTAGE Ca	PERCENTAGE Mg	PERCENTAGE K	SO ₄ PRESENT
	%	%	%	
None	0.091	0.140	1.23	Yes
Soaked 5 hours	0.083			Yes
Control 15 minutes	0.083			Yes
Electrodialysis 15 minutes	0.085			Yes
Control 1 hour	0.091	0.140	1.11	Yes
Electrodialysis 1 hour	0.073	0.130	0.712	Yes
Control 2 hours	0.085	0.140	1.13	Yes
Electrodialysis 2 hours	0.067	0.115	0.545	No
Control 3 hours	0.087		1.04	Yes
Electrodialysis 3 hours	0.060	0.095	0.413	No

Either the sulphate of the seeds or the fraction of the seeds which goes to form sulphate on ashing was lost in two or more hours of electro-dialysis. Sulphate was not determined quantitatively but its presence was tested for by the use of barium chloride.

ASCORBIC ACID IN THE GERMINATING SEEDS

Ascorbic acid in germinating Alaska pea seeds was determined on the second, third, and fourth days after treatment. These seeds were electro-dialyzed for two hours at 80 volts. After treatment, the seeds were kept in flannel rolls moistened with tap water until the time for determination of the ascorbic acid. Amounts of ascorbic acid in the seeds after different lengths of time are shown in table X.

From the results of the analyses presented in table X it can be seen that electro-dialysis is followed by low ascorbic acid content of germinating

pea seeds during the early stages of germination. On the fourth day after treatment the ascorbic acid content of the electrodialyzed seeds is almost as high as that of the control lots. This appears significant when it is considered that the control lots have a germination of 90 per cent. while the electrodialyzed seeds have about 35 per cent. germination.

TABLE X

ASCORBIC ACID CONTENT OF ALASKA PEA SEEDS AFTER ELECTRODIALYSIS
FOR TWO HOURS AT 80 VOLTS

SAMPLE NUMBER	MG. ASCORBIC ACID PER GRAM OF TISSUE					
	2 DAYS		3 DAYS		4 DAYS	
	CONTROL	ELECTRO- DIALYSIS	CONTROL	ELECTRO- DIALYSIS	CONTROL	ELECTRO- DIALYSIS
1	0.18	0.03	0.25	0.10	0.32	0.26
2	0.16	0.02	0.30	0.11	0.27	0.22

Discussion

The most obvious result of electrodialysis on the seeds used in this work was the reduction of the percentage of germination. By reference to tables II and III it will be noticed that electrodialysis of Alaska pea seeds at 80 volts has a more pronounced effect on their germination than has electro-dialysis at 110 volts. No explanation can be offered for this reaction to the electro-dialytic process. As electro-dialysis takes place at greater rates with increases in voltage, it would appear that the effect on germination must be caused in part by something other than removal of electrolytes from the seeds. It is also difficult to see any close relationship between the removal of calcium, magnesium, and potassium and the percentage germination in the Laxton's Progress pea seeds. Loss of germination is very marked before any great amounts of calcium, magnesium, or potassium are removed from the seeds.

Respiration of Laxton's Progress pea seeds, as measured by their production of carbon dioxide, is greatly affected by electro-dialysis at 150 volts. As mentioned before, lowering of carbon dioxide production does not occur to the same extent as germination is lowered for any group of seeds. Respiration seems to bear out the same relation to removal of minerals as does germination to removal of minerals. Carbon dioxide production falls off to a great extent before any appreciable loss of magnesium or calcium takes place. Potassium, however, is lost early in the electro-dialysis process and may possibly be associated with decrease in respiratory activity. Although carbon dioxide production is less following electro-dialysis, it is interesting to note that seeds which fail to germinate because of the treatment still give off carbon dioxide.

Calcium, magnesium, and potassium are all lost to varying extents from Laxton's Progress pea seeds during the course of electro-dialysis. Although

some potassium was lost from the control lots of these seeds while soaking in distilled water, no appreciable amounts of calcium and magnesium were lost from the control lots. The difficulty found in the ashing of seeds electro-dialyzed for an hour or more may have been caused by a lesser amount of mineral matter in the seeds. A phenomenon similar to this is encountered in the study of burning qualities of tobacco. HALEY and OLSON (2) suggest that good combustion of cigar-leaf tobacco is associated with potassium compounds which give rise to potassium carbonate during burning, and that the presence of such compounds may serve to intensify the catalytic effects of iron in this process. It has also been reported by HALEY, NASSET, and OLSON (1) that in practically all cases there is a parallelism between the burning qualities of tobacco and the water-soluble alkalinity of the ash.

It is demonstrated that a greater quantity of base than acid is removed from the seeds during electrodialysis. This is shown by the amount of acid necessary to neutralize the cathode chamber, being much more than equivalent to the amount of alkali necessary to neutralize the anode chamber after dialysis. Possibly this may give some indication of the state of cations in protoplasm. From this it would appear that the cations are either adsorbed on surfaces or are in combination with anions which cannot penetrate the membranes through which they would have to pass in order to reach the anode chamber. When adsorbed cations are removed from soils by electrodialysis, they are replaced by hydrogen ions from the water in which electrodialysis takes place. It is very probable that a similar reaction takes place in seeds which lose cations through electrodialysis.

The effect of electrodialysis on the ascorbic acid content of Alaska pea seeds electro-dialyzed for two hours at 80 volts is very marked. During the early stages of germination this treatment was followed by very low amounts of ascorbic acid in the seeds. By the end of the fourth day after treatment, however, the electro-dialyzed seeds contained almost as much ascorbic acid as the control lots. This seems significant in view of the fact that germination in the treated seeds was much lower than in the control lots.

Summary

1. Germination of Laxton's Progress and Alaska pea seeds, and red kidney beans was markedly decreased by electrodialysis of the seeds.
2. Longer periods of electrodialysis were accompanied with lesser percentages of germination.
3. Production of carbon dioxide was studied in Laxton's Progress pea seeds after they had been electro-dialyzed, and it was found that this treatment had a great inhibiting effect on subsequent carbon dioxide production.
4. Calcium, magnesium, and potassium were all lost at a much greater rate from electro-dialyzed Laxton's Progress pea seeds than from control lots.
5. Cations removed from all the lots of seeds studied were more than equivalent to the removed anions.

6. In the ash of Laxton's Progress pea seeds electrodialyzed two hours or more at 150 volts there was no sulphate ion present.

7. Electrodialysis of Alaska peas for two hours at 80 volts was followed by a very low ascorbic acid content during the early stages of germination. By the end of the fourth day the electrodialyzed seeds contained nearly as much ascorbic acid as the controls.

This work was done partly in the Department of Horticulture of the Pennsylvania State College, and the remainder in the Department of Botany of the University of Chicago. The writer wishes to express his thanks to Dr. C. A. SHULL, Dr. W. B. MACK, and Dr. E. C. DUNKLE for help rendered during the course of the work. Dr. CHARLES L. HAMNER did the major portion of the work in the ascorbic acid determinations.

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